**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**
**REQUEST FOR FILING APPLICATION UNDER 37 CFR 53(b)**
**WITHOUT FILING FEE OR EXECUTED INVENTOR'S DECLARATION**



Assistant Commissioner for Patents
Washington, DC 20231



Atty. Dkt. 555-56

Date: March 27, 2000

Sir:

This is a request for filing a new PATENT APPLICATION under Rule 53(b) entitled:
METHOD OF TREATING ATHEROSCLEROSIS
without a filing fee and/or without an executed inventor's oath/declaration.
This application is made by the below identified inventor(s). Attached hereto are the following papers:

☒ An abstract together with
**17** pages of specification and claims including
**7** numbered claims and also attached is/are
**1** sheet of accompanying drawing.
☐ This application is based on the following prior foreign application(s):

| **Application No.** | **Country** | **Filing Date** |
|---|---|---|

respectively, the entire content of which is hereby incorporated by reference in this application, and priority is hereby claimed therefrom.

☒ This application is based on the following prior provisional application(s):

| **Application No.** | **Filing Date** |
|---|---|
| **60/126,407** | **26 March 1999** |

respectively, the entire content of which is hereby incorporated by reference in this application, and priority is hereby claimed therefrom.

☐ Certified copy/ies of foreign applications attached.
☐ This application is a ☐ continuation/☐ division/☐ continuation-in-part of application Serial No. , filed .
☐ Please amend the specification by inserting before the first line: --This application is a ☐ continuation/☐ division/ ☐ continuation-in-part of application Serial No. , filed , the entire content of which is hereby incorporated by reference in this application.--
☐ Please amend the specification by inserting before the first line: --This is a continuation of PCT application No. , filed , the entire content of which is hereby incorporated by reference in this application.--
☐ Please amend the specification by inserting before the first line: --This application claims the benefit of U.S. Provisional Application No. **60/126,407**, filed **26 March 1999**, the entire content of which is hereby incorporated by reference in this application.--
☐ Preliminary amendment to claims (attached hereto), to be entered before calculation of the fee.
☐ Also attached.

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# *U.S. PATENT APPLICATION*

*Inventor(s):* Alan D. Schreiber

*Invention:* METHOD OF TREATING ATHEROSCLEROSIS

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# *SPECIFICATION*

# METHOD OF TREATING ATHEROSCLEROSIS

This application claims priority from Prov. App. No. 60/126,407, filed March 26, 1999. The entire contents of the provisional application is incorporated herein by reference.

## TECHNICAL FIELD

The present invention relates to a method of treating atherosclerosis, and to compounds and compositions suitable for use in such a method.

## BACKGROUND

Delayed development of coronary artery disease in premenopausal women, compared to that of age-matched men, was noted over sixty years ago (Levy et al, J. Am. MedAssoc. 107:97-102 (1936), Master et al, Arch. Int. Med. 64:767-786 (1939)). Despite numerous reports addressing the antiatherogenic effect of estrogen in premenopausal women and in postmenopausal women on estrogen replacement therapy, the mechanism of this protective effect and its relationship to alteration in serum lipids is still unclear.

Even less is known about the effect of progesterone on the development of atherosclerosis, either alone or in combination with estrogen. Conflicting clinical and experimental data suggest that the effect of exogenous progestogens on the

development of coronary artery disease ranges from adverse (Hirvonen et al, N. Engl. J. Med. 304:560-563 (1981)) to null (Adams et al, Arteriosclerosis 10:19051 (19090)) to potentially desirable (Haarbo et al, Am. J. Med. 90:584-589 (1991)) to favorable (Alexandersen et al, Arterioscler Thromb. Vasc. Biol. 18:902-907 (1998)) when combined with estriol treatment. A favorable effect of progesterone treatment alone is not believed to have been documented in a clinical study.

Various animal models used to study the effects of sex steroids on the development of atherosclerosis have demonstrated beneficial results associated with exogenous estrogen with or without progesterone in chickens (Pick et al, Circulation 6:276-280 (1952), Pick et al, Circulation 4:468 (1951)), mice (Sullivan et al, J. Clin. Invest. 96:2482-2488 (1995)), rabbits (Kushwaha et al, Metabolism 30:359-366 (1981), Fischer et al, Atherosclerosis 54:177-185 (1985), Haarbo et al, J. Clin. Invest. 87:1274-1279 (1991), Hanke et al, Circulation 94:175-181 (1996), Holm et al, Circulation 98:2731-2737 (1998)), monkeys (Wagner et al, J. Clin. Invest. 88:1995-2002 (1991)), and baboons (Kushwaha et al, Arterioscler. Thromb. 11:23-31 (1991)). Spagnoli and coworkers (Spagnoli et al, Atherosclerosis 82:27-36 (1990)) reported for the first time that high-dose synthetic progestogens alone inhibited atherosclerotic plaque formation in female rabbits fed a cholesterol-

enriched diet and concluded that the protective effect was only partly due to alteration in serum cholesterol.

The present invention results, at least in part, from the observation that aortic atherosclerotic plaque load can be decreased in hypercholesterolemic male rabbits with exogenous progesterone as well as with estriol, the decreases in plaque load being independent of alterations in serum lipid levels.

## SUMMARY OF THE INVENTION

The present invention relates to a method of treating atherosclerosis. The method comprises administering to a patient in need thereof an effective amount of a progestational agent that has a minimal effect on sex organs or a non-steroidal compound that inhibits macrophages or macrophage function.

Objects and advantages of the present invention will be clear from the description that follows.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the stucture of haloperidol, SCH-23390 and sulpiride.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of treating atherosclerosis (including atherosclerotic cardiovascular disease and stroke) in a mammal (e.g., a human). The method comprises administering to a mammal suffering from atherosclerosis an effective amount of a progestational agent that has a minimal effect on sex organs (e.g., less of an effect that medroxyprogesterone acetate) or a non-steroidal compound that inhibits macrophages or macrophage function (e.g., that inhibits macrophage Fc receptors).

Progestational agents suitable for use in the invention include the progesterone analogs of USP 4,902,681 and 4,908,358. A preferred such agent is 17-hydroxyprogesterone.

Non-steroidal compounds suitable for use in the present invention include benzazepines and butyrophenones, and pharmaceutically acceptable salts thereof. Benzothiophenes described in USP 5,075,321 can also be used. SCH-23390 is an example of a suitable benzazepine and haloperidol is an example of a suitable butyrophenone (see Figure 1). Analogues of, for example, SCH-23390 or haloperidol, that is, compounds having a similar structure or similar binding affinity for dopamine receptors or comparable capacity to antagonize or stimulate dopamine

receptors, can also be used. Structurally similar structures include those wherein an alternative halogen is present as a substituent (e.g., F rather than Cl in the case of SCH-23390) or an alternative $C_1$-$C_4$ alkyl substituent is present (e.g., $-CH_2CH_3$ rather than $-CH_3$ in the case of SCH-23390). Benzazepines and butyrophenones have been shown to bind dopamine receptors (SCH-23390 is a $D_1$ antagonist while haloperidol is a $D_2$ antagonist (Sunahara et al, Br. J. Psych. 163(suppl. 22):31 (1993)). Other $D_1$ and $D_2$ antagonists can be used in accordance with the present method.

Compounds of the invention can be formulated into pharmaceutical compositions suitable for administration via a variety of routes. For example, the compositions can be suitable for oral, rectal, intravenous, or parenteral administration. The compounds can also be formulated so as to be suitable for administration via inhalation. Compositions suitable for such forms of administration are, advantageously, sterile.

Depending on the intended mode of administration, the pharmaceutical composition can be in the form of solid, semi-solid or liquid dosage form, such as, for example, a tablet, pill, capsule, powder, liquid, suspension, or the like, preferably in unit dosage form suitable for single administration of a precise dosage. The pharmaceutical composition can include a

conventional pharmaceutical carrier or excipient and a compound according to the invention as an active ingredient. In addition, it can include other medicinal or pharmaceutical agents, carriers, adjuvants, etc.

For oral administration, which is preferred, the pharmaceutical composition can take the form of a solution, suspension, tablet, pill, capsule, powder, sustained release formulation or the like.

For solid pharmaceutical compositions, conventional non-toxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. Liquid pharmaceutically administrable compositions can be prepared by dissolving or dispersing, or otherwise preparing a compound according to this invention and mixing it optionally with a pharmaceutical adjuvant in a carrier, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension.

Methods of preparing compositions with a certain amount of active ingredient are known, or will be apparent, to those skilled in this art. For example, see Remington's Pharmaceutical Sciences., Mack Pub. Co., Easter, Pa., 15th Ed. (1975).

Dosage regimens suitable for use in the present

invention can be selected so as to achieve the clinical response (e.g., reduction in atherosclerosis plaque load) sought. The doses used will vary depending, for example, on the specific compound employed, the status of the patient and the route of administration. While optimum doses can be readily determined, dosages will generally be selected such that local blood concentrations of 100 to 200,000 pg/ml are achieved, 1,000 to 100,000 pg/ml being preferred, and 10,000 to 100,000 pg/ml being most preferred.

The following non-limiting Examples describes certain aspects of the invention in greater detail.

EXAMPLE 1

EXPERIMENTAL DETAILS

Male New Zealand White rabbits (3.2-4.9 pounds each) were fed a 0.5% cholesterol chow diet for 12 weeks and randomly assigned to five groups (n=6 each): one control group (CG) and four groups treated with estriol (E), haloperidol (H), low-dose 17-hydroxyprogesterone (LDP), or high-dose 17-hydroxyprogesterone (HDP). Serum cholesterol (C) and triglyceride (T) were measured before and after the treatment period in all groups; serum progesterone was determined only in the LDP and HDP groups. After the treatment period, rabbits were euthanized for

histomorphometric analysis of aortic plaque load. The rabbits were treated according to the Animal Welfare Act specifications.

Drug Administration

Vehicle only (CG) and vehicle plus steroid (treated groups) were administered daily subcutaneously. Steroid doses were as follows: estriol, 1mg/kg; haloperidol, 1mg/kg for 2 weeks, 10 mg/kg for 2 weeks, and 5 mg/kg for 8 weeks; low-dose 17-hydroxyprogesterone, 10 mg/kg; high-dose 17-hydroxyprogesterone, 90 mg/kg. The dose of haloperidol was reduced from 10 mg/kg because of the rabbits' poor tolerance of the higher dose.

Histomorphometric Analysis

Five-micron, serial histologic sections (15 each) of 27 ascending aortas were cut from paraffin blocks, stained with hematoxylin and eosin (H&E) and were studied by light microscopy. Images of tissue sections were captured to a Power Macintosh 7300/200 computer by a Hitachi 3-CCD Color Camera (model HV-C20) attached to a Nikon Eclipse E600 microscope. With digital imaging (IPLap Spectrum, Signal Analytics Corporation, Vienna, VA), they were then analyzed by manual color segmentation by tracting the endothelial surface (intima), internal elastic lamina, and external elastic lamina of each vessel. From

segmented images, intimal and medial areas were computed, allowing calculation of intima to media ratios. Plaque load for each aorta was defined as the ratio of intimal area to medial area (I/M).

Statistical Analysis

Serum lipid levels and I/M were expressed as the mean+SEM for all animals. The changes in lipid levels before and after the treatment period were compared using paired t-tests. The differences between the treatment groups and controls were compared using analysis of variance (ANOVA) techniques. Repeated Measures ANOVA was used to compare the difference in plaque loads among the five groups. Multiple regression models were used to compare the differences in plaque loads between the four treatment groups and the control group, adjusting for the difference in cholesterol and triglyceride levels. $P<0.05$ was considered statistically significant.

RESULTS

Alteration of Serum Lipids

Over the treatment period, mean serum C increased significantly in all groups. In the HDP group, the increase in serum C (cholesterol) was even greater than that of the CG. Serum T (triglyceride) decreased slightly in the E group but significantly increased in the other groups. Post-treatment serum P levels were

significantly higher than baseline levels in the LDP and HDP groups. These data are summarized in Table 1.

Gross and Microscopic Findings

After 91-100 days of treatment, twenty-seven rabbits were euthanized for histomorphometric analysis of aortic plaque load. One rabbit in the E group died after 18 days of treatment, and two rabbits in he H group were sacrificed at 39 and 71 days because of their failure to thrive.

Gross examination of the aortas revealed decreased atherosclerosis in the progesterone and estrogen treated animals. With the control animals, there was about 42% occlusion. With the low dose progesterone there was 6% occlusion and with the high dose progesterone there was 0% occlusion evident.

Fifteen stained serial histologic sections of ascending aorta from each of the twenty-seven surviving rabbits were examined by light microscopy. Atherosclerotic plaques in all specimens revealed variably dense aggregates of macrophage foam cells and α-actin positive smooth muscle cells in a thickened intimal matrix. Rounded foam cells were commonly seen straddling both sides of the focally degenerated internal elastic lamina.

Analysis of Aortic Plaque Loads

Study of the mean I/M for each group, determined by computerized image analysis, revealed significant decreases in all four treatment groups ($P<0.001$ for E, LDP and HDP, and $P=0.02$ for H) when compared to the CG, as shown in Table 2. As further shown in Table 3, both LDP and HDP decreased the atherosclerotic plaque.

Even though the change in serum C and T levels did not have strong relationships with I/M, the values were quite different among the five groups. The group differences were examined, adjusting for the changes in serum C and T levels using a multiple regression model. The aortic plaque load remained lower in all four groups that that of the controls, E ($P=0.052$), H ($P=0.069$), LDP ($P=0.026$), HDP ($P=0.014$), after controlling for the changes in serum C and T levels; the plaque load difference approached statistical significance in the E group and reached the significance level in the HDP group.

Table 1. Quantitative Serum Lipid Analysis

| | | Treatment groups | | | | |
|---|---|---|---|---|---|---|
| | | CG | E | H | LDP | HDP |
| | n: | 5 | 5 | 4 | 6 | 5 |
| Cholesterol (pg/dl) | B: | 49 | 83.6 | 64.25 | 50.33 | 54 |
| | A: | 1421.6 | 1062.8 | 1251.2 | 1441.5 | 2282.67 |
| | $\Delta$=A-B[1]: | 1372.6 | 979.2 | 1187 | 1391.17 | 2228.67 |
| | $\Delta/\Delta_{CG}$: | 1.00 | 0.71 | 0.86 | 1.01 | 1.62[3] |
| Triglyceride (pg/dl) | B: | 59.67 | 111 | 86.25 | 71 | 75.8 |
| | A: | 543.6 | 96.8 | 476.75 | 560.83 | 1035.33 |
| | $\Delta$=A-B[2]: | 483.93 | -14.2 | 390.5 | 489.83 | 959.53 |
| | $\Delta/\Delta_{CG}$: | 1.00 | -0.03 | 0.81 | 1.01 | 1.98 |
| Progesterone (ng/ml) | B: | — | — | — | 0.28 | 0.2 |
| | A: | — | — | — | 3.03 | 6.48 |
| | $\Delta$=A-B[1]: | — | — | — | 2.75 | 6.28 |

B--Before treatment; A--After treatment

1--All changes with $P<0.05$

2--All changes with $P<0.05$, except for the E group

3--$P<0.05$ when compared to the CG

Table 2. Morphometric Analysis of Plaque Load

| | Treatment groups | | | | |
|---|---|---|---|---|---|
| | CG | E | H | LDP | HDP |
| n: | 6 | 5 | 4 | 6 | 6 |
| Mean I/M: | 0.81 | 0.02 | 0.29 | 0.42 | 0.47 |
| STD: | 0.43 | 0.04 | 0.39 | 0.21 | 0.25 |
| $(I/M)/(I/M)_{CG}$: | 1.00 | 0.02 | 0.29 | 0.42 | 0.47 |
| P value*: | — | 0.0001 | 0.0198 | 0.0001 | 0.0008 |

*when compared to the control group

TABLE 3

ATHEROSCLEROTIC PLAQUE INHIBITION BY 17-OH PROGESTERONE

PLAQUE SIZE IN HYPERCHOLESTEROLEMIC RABBITS

VEHICLE (RABBIT#)

| 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|
| 0.31 | 0.90 | 1.57 | 1.32 | 0.69 | 2.13 |

MEAN = 1.15

LOW DOSE (RABBIT#) (10 mg/kg)

| 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|
| 0.66 | 0.30 | 0.84 | 0.85 | 0.14 | 0.52 |

MEAN = 0.55

HIGH DOSE (RABBIT#) (90 mg/kg)

| 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|
| 1.37 | 0.11 | 0.33 | 0.23 | 0.18 | 0.02 |

MEAN = 0.37

EXAMPLE 2

Inhibition of Human Monocyte/Macrophage Fcγ Receptor Mediated Phagocytosis

The agents to be tested (haloperidol, SCH-23390 and sulpiride) were incubated with human monocytes isolated using lymphocyte separation medium. Incubation was for 90 min at 37°C. Buffer (PBS) treated cells served as a control. At the end of the incubation period, monocytes were exposed to IgG-sensitized cells in accordance with the phagocytic assay of Indik et al, Blood 86:4389 (1995). The results are as shown in Table 4.

Table 4

| | %Phagocytic Cells[1] | PI[2] |
|---|---|---|
| Buffer (PBS) treated control | 42% | 105 |
| Haloperidol (50 μM PBS/methanol) | 0% | 0 |
| SCH-23390 (50 μM PBS/methanol) | 16% | 38 |
| Sulpiride (500 μg/ml PBS/methanol) | 59% | 232 |

[1] % Phagocytic cells = Percent of monocytes/macrophages phagocytosing IgG sensitized cells

[2] PI = Phagocytic index = The number of ingested IgG-sensitized cells per 100 monocytes.

No change in expression of the monocyte Fcγ receptor FcγRI, FcγRII or FcγRIIIA was noted using flow cytometry analysis.

* * *

All documents cited above are hereby incorporated in their entirety by reference.

One skilled in the art will appreciate from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention.

WHAT IS CLAIMED IS:

1. A method of treating atherosclerosis in a mammal comprising administering to a mammal in need of such treatment: i) a progestational agent that has a minimal effect on the sex organs of said mammal or ii) a non-steoidal compound that inhibits macrophages or macrophage function, wherein said agent or said compound is administered in an amount sufficient to effect said treatment.

2. The method according to claim 1 wherein said progestational agent is administered and said agent has less of an effect than medroxyprogesterone acetate on said sex organs.

3. The method according to claim 1 wherein said non-steroidal compound is administered.

4. The method according to claim 3 wherein said non-steroidal compound inhibits macrophage Fc receptors.

5. The method according to claim 1 wherein 17-hydroxyprogesterone is administered.

6. The method according to claim 1 wherein said non-steroidal compound is administered and said non-steroidal compound is a benzazepine or a butyrophenone.

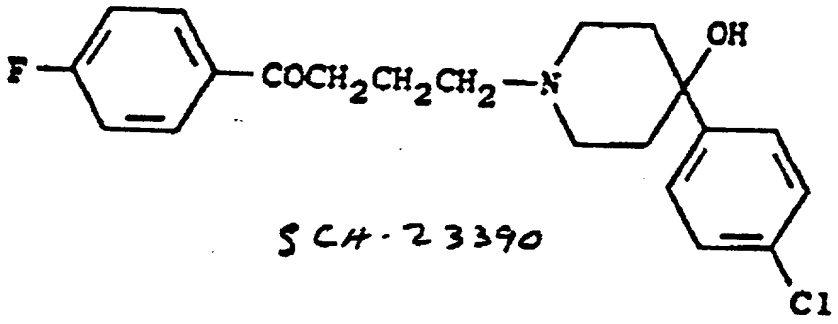
7. The method according to claim 6 wherein SCH-23390 or haloperidol is administered.

## ABSTRACT OF THE DISCLOSURE

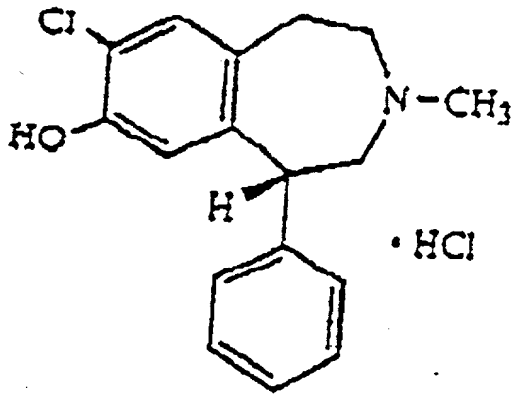
The present invention relates to a method of treating atherosclerosis, and to compounds and compositions suitable for use in such a method.

Fig. 1

Haloperidol




SCH-23390




Sulpiride